

THE ROCKEFELLER INSTITUTE  
FOR MEDICAL RESEARCH

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NEW YORK 21, N. Y.

December 8, 1952

Dear Josh:

This is to answer yours of the 24th and 25th. I don't think any enclosure with the reprints is necessary but thanks for asking anyway.

Re: resspreading experiments. There are few details I can add as illness and the pressure of other work has kept me from it. The culture used was SW-351 (Gal<sup>+</sup> S<sup>r</sup>) and the phage PLT-22/7S<sup>r</sup>. I have since done a reconstruction experiment which essentially invalidates the conclusions with S<sup>r</sup> as the clones don't increase in time. This being due to inhibition of colony formation by the heavy curds of debris etc rather than differential growth rate. The Gal story remains intact. The mutant is sufficiently stable that thus far I've not had to correct for spontaneous mutants in time. The experiment is not easily done in broth as one can't grow populations of sufficient size, in single steps anyway, which would have sufficient number of transductions per plating sample. However in order to avoid the mess with SM and still study the character and also Aux-prot transductions I shall set up a primitive chemostat and try the experiment in broth. So far there has been little difference in the growth rates on EMB and NA. The immediate stimulus for this experiment was Hotchkiss' finding of a similar delay with S<sup>r</sup> transformations. He believes this to be inhibitional not segregational which as you say will be difficult to prove. He now has a mannitol transformation.

\* Re: SW-541 and 666. I merely streaked out the 541 you sent me. The rest was pure speculation. Have separated the + and S components which are stable and prepared FA from each to do the genetic tests. The full ~~plus~~ pluses which you mention but which I've not seen made me hopeful for another linked transduction.

Re; lytic variants. I have thus far defined a lytic variant as one that does not induce lysogenicity but does select resistant strains which thus far have all been roughs. In this sense lytic viruses transduce. I've not yet tested whether they kill sensitive cells after adsorption which is probably the best criterion.

Your experiment with 666 and lysogenicity is most useful in that it supports FA-phage and also transduction need not result in phage infection.

Why should the ~~receptor~~ receptor cells be more contaminated with alternate phases than the donor cells (FA II lacking phase I components)?

Apparently some time last summer you most flatteringly told Szybalski that it would take me about three months to explain Schneiders problem. The three months are now up and I shall report the progress to date.

The problem was this. He had two strains of typhimurium which could be differentiated by no means (antigenicity, phagocytic index etc) other than one was virulent (single cell will kill) and the other avirulent (10<sup>5</sup> cells will kill). If he injected 1000 cells of each simultaneously all mice die. If he first injected with avirulent and then after two days with virulent the mice had a higher chance of survival especially if on a natural diet rather than a synthetic (40% difference). These experiments have since been repeated with a marked virulent strain (marking the avirulent might have produced some secondary affect~~ed~~ we could not have assayed). The mice were sacrificed in time and the *spleens*

\* all transductions are slow  
50 clones of each tested

ground and plated. With the mixed challenge at zero days the avirulents go way ahead establishing a ratio of greater than 100 to 1 and achieve titers of  $10^5$  per spleen by five days when the mice start to die. Those mice on the natural diet have higher titers of virulents and correspondingly die sooner. With the two day post challenge (avirulents are a few thousand per spleen at this time) the avirulents with the synthetic diet establish themselves in the spleen in 24 hours but much depressed when compared to the one day mixed challenge. On the natural diet however it takes from 48-72 hours before the virulents appear similarly much depressed. By five days after secondary challenge the virulents on the ~~second~~ <sup>synthetic</sup> diet outnumber the avirulents but are rising very slowly. At the same time on the natural diet some mice have equal numbers of the two and others the virulents are increasing rapidly mirroring the mixed challenge on this diet.. All survivors will soon be sacrificed to see what has happened there. We now know what happens in vivo but not why it happens. It also give us a smaller mouse assay for this dietary factor, previously taking forty mice to test a fractionation now about four so the resolution of the material won't require tons of extract.

Sincerely,

*Norton*

Norton